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Effect of *Streptococcus pneumoniae* Infection in Rats on Hepatic Water Content, Electrolyte Concentration, and Chemical Composition

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SUMMARY

Total hepatic water content, dry weight, protein, lipid, carbohydrate, RNA, DNA, and electrolyte concentrations were determined in control and *Streptococcus pneumoniae*-infected rats. During infection, there was a significant ($P = 0.001$) increase in total liver weight. This increase was the result of increased total liver water content and increased total liver dry weight. Intracellular water content, diameter of hepatocytes, and all measured constituents of hepatic dry weight, excluding DNA, increased significantly (P varied from 0.05–0.001) during infection. Concentrations of liver Na^+ and Cl^- increased significantly (P varied from 0.05–0.005), whereas the concentration of liver K^+ decreased significantly ($P = 0.01$). Seemingly, there is an enlargement of hepatocytes due to increased intracellular water and increased dry weight during pneumococcal infection in the rat. The mechanism of these liver changes may be due, in part, to a shift of Na^+ and K^+ across liver cell plasma membranes.

Inoculation of rats with *Streptococcus pneumoniae* results in an acute systemic extracellular infection and marked alterations in systemic host metabolism. Metabolic changes include increased uptake of zinc[1] and amino acids[2–4] by the liver, and altered hepatic protein,[3,5–7] nucleic acid,[7,8] carbohydrate,[9] and lipid metabolism.[10,11] Increased synthesis, transport, and secretion of plasma proteins by the liver during *S pneumoniae* infection have also been reported.[12] In addition, *S pneumoniae* infection alters hepatic enzyme composition,[5,11] as well as the number[13] and equilibrium density[14] of certain hepatic cellular organelles.

During experiments to determine effects of *S pneumoniae* infection on the structure and function of liver cell membranes in the rat, a marked increase in the size and weight of the liver was observed during infection. The present study was designed to determine the mechanism of this increased liver weight. These determinations were necessary to evaluate biochemical changes that occur in the liver during infection.

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Materials and Methods

Animals—Male albino rats (260 to 280 g) of the Sprague-Dawley strain [Crl:COBS(SD) BR][a] were maintained on laboratory animal feed[b] with tap water ad libitum. All rats were acclimated to a 12-hour day-night cycle (light cycle from 2:00 PM to 2:00 AM) for 12 days before experimentation to standardize circadian variations. Rats were inoculated subcutaneously with 3×10^5 to 6×10^5 heat-killed (control) or virulent (infected) colony-forming units of *S pneumoniae*, serotype I, A-5 strain organisms (this dose will consistently kill approximately 80% of the inoculated rats by postinoculation day 5, with a mean time to death of 66 hours.) After inoculation, all rats were fasted, but were allowed access to water, and were euthanatized at 8:00 AM, 40 hours after inoculation, a time corresponding to the midpoint of the night cycle. Fasting was necessary because infected rats were anorectic.

Analysis of Nonperfused Liver Samples—Rats (n = 12) were exsanguinated; livers were removed, blotted gently, and weighed. For determination of water content and dry weight, 1 g of liver was weighed on an analytical balance and was placed in an oven at 100 C for 3 days until constant weight was achieved.[15,16] Tissue electrolytes (Na^+ and K^+) were determined on another portion of liver by flame photometry[15,17] after tissue homogenization in 10% trichloroacetic acid (1:10, w/v). Tissue Cl^- was extracted with distilled water and was analyzed with a chloridometer.[18] Intracellular and extracellular water and electrolyte distributions were calculated,[19] using a Donnan factor of 0.95.[20]

Analysis of Perfused Liver Samples—Rats (n = 10) were anesthetized with a mixture of droperidol and fentanyl[c] IM (0.1 ml/200 g of body weight), and livers were perfused in situ with normal saline solution[d] according to the following procedure: A 19-gauge needle was placed into the portal vein and was secured with surgical thread, and the perfusion was started at a rate of 55 ml/minute, using a peristaltic pump. The descending aorta and hepatic vein were then severed in the lumbar region. After complete perfusion (approx 2 minutes), the liver was removed and weighed after gentle blotting on a gauze pad. For the determination of protein, RNA, DNA, and carbohydrate, 1 g of liver was cut into pieces with scissors in 9 ml of ice-cold saline solution and was minced further in a homogenizer[e] at a setting of 400 rpm. Water content and dry weight were determined as described for nonperfused liver samples. The remaining portion of liver was used for

[a] Charles River Breeding Laboratories, Wilmington, Mass.
[b] Wayne Lab Chow, Chicago, Ill.
[c] Innovar-Vet, Pitman-Moore Inc, Washington Crossing, NJ.
[d] 0.9% NaCl, Abbott Laboratories, North Chicago, Ill.
[e] Model K 43 mechanical homogenizer, TRI-R Instruments Inc, Rockville Centre, NY.

total lipid extraction[21] and subsequent determination of total lipids.[22]

Histologic Procedures—After rats (n = 6) were anesthetized,[c] the thoracic cavities were opened, and a 19-gauge needle on an infusion line was inserted into the left ventricle of the heart. An infusion of 6% (w/v) dextran in saline solution[d] was started, followed by excision of the right auricular appendage. The infusion was allowed to continue until the effluent from the right atrium was clear. The perfusate was then replaced with 10% formalin, and the infusion was allowed to continue for approximately 5 minutes. The liver was removed and weighed, and several representative sections were taken for subsequent histologic examination.

Sections (4 μm thick) were prepared, using standard histologic techniques and were stained with hematoxylin and eosin (H&E) or Gram-Weigert's stain. The Gram-Weigert-stained sections were screened for the presence of typical, lancet-shaped, gram-positive diplococci. Morphometric measurements were performed on the H&E-stained sections, using an ocular micrometer that had been calibrated against a stage micrometer. All measurements were performed, using the oil immersion objective (1,000×). The diameters of randomly selected hepatocytes containing well-defined nuclei and cytoplasmic borders were measured. Cells that were multinucleated or did not contain nuclei in the plane of the section were not measured. A total of 25 cells was measured from at least 3 coded sections representing different areas of the liver. The code was not broken until all measurements had been completed.

Analytical Procedures—Protein was determined by the method of Lowry et al,[23] using bovine serum albumin[f] as a standard. The RNA was assayed by the orcinol method,[24] with the yeast RNA[g] as a standard. The DNA was assayed by the method of Burton,[25] using calf thymus DNA[f] as a standard. Lipid was extracted by the method of Folch et al.[21] Carbohydrate was assayed by the method of Dubois et al,[26] using sucrose as a standard.

Determination of Plasma Water—Plasma water was determined by a gravinometric method. Immediately after separation from RBC, a plasma sample was pipetted into a weighed vial. The vial was placed in an oven at 100 C. After 2 days, the vial was reweighed and then was weighed daily until a constant weight was obtained.

Statistics—Group mean values were compared by Student's *t* test, and the difference between the 2 means was considered significant at $P < 0.05$.

Results

The effects of *S pneumoniae* infection on the water content, dry weight, and chemical composition of rat liver are shown (Table 1). There were significant increases in water and RNA content and a significant decrease in the dry weight per gram of wet liver. Significant changes in protein, lipid, RNA, carbohydrate, or DNA concentrations were not observed in the livers of infected rats compared with concentrations in control rats.

There was a significant increase in the total liver weight during *S pneumoniae* infection (Table 2). When the results shown in Table 1 are expressed for the total liver, it was evident that the increase in liver weight observed during infection was the result of a significant increase in total liver water, but also in total liver dry weight. Also, the percentage of the liver weight that was water increased slightly but significantly. The percentage of the liver weight that was dry weight decreased slightly but significantly.

[c] Sigma Chemical Co, St. Louis, Mo.
[g] Yeast RNA standard, Calbiochem Inc, Gaithersburg, Md.

TABLE 1—Effect of *S pneumoniae* Infection on Hepatic Water Content, Dry Weight, and Chemical Composition of Ten Rats

Variable (mg)	Controls (n = 5)	Infected (n = 5)	*P*
Water content	764 ± 3	782 ± 5	< 0.01
Dry weight	237 ± 3	219 ± 4	< 0.005
Protein	132 ± 3	130 ± 3	NS
Lipid	39 ± 3	40 ± 3	NS
RNA	12.2 ± 0.8	14.4 ± 0.8	< 0.025
Carbohydrate	1.4 ± 0.03	1.4 ± 0.03	NS
DNA	9 ± 0.8	10 ± 0.6	NS

Data expressed as mean ± SE of mg/g of liver (wet weight); NS = not significant. n = No. of rats.

TABLE 2—Effect of *S pneumoniae* Infection on Total Liver Weight, Water Content, and Dry Weight of Ten Rats

Total liver	Controls (n = 5)	Infected (n = 5)	*P*
Weight (g)	9.58 ± 0.23	12.25 ± 0.36	< 0.001
Water content (g)	7.32 ± 0.20 (76.4 ± 0.32)	9.56 ± 0.32 (78.0 ± 0.52)	< 0.001 < 0.025
Dry weight (g)	2.27 ± 0.04 (23.7 ± 0.85)	2.68 ± 0.07 (21.9 ± 0.42)	< 0.001 < 0.005

Data expressed as mean ± SE. No. in parentheses are percentages of total liver weight. Values for total water content and dry weight were obtained by multiplying results in Table 1 by total liver weights. n = No. of rats.

Each major constituent of the dry weight was also measured. Every constituent, with the exception of DNA, increased significantly (Table 3). The greatest contribution to the increase in dry weight was protein, followed by lipid, RNA, and carbohydrate. The percentage of the dry weight made up by each of these constituents decreases in the same order. As shown in Table 3, the percentage of the dry weight that was RNA and carbohydrate increased significantly.

The mean value of total, intracellular, and extracellular water content, as well as Na^+, Cl^-, and K^+ concentrations, in the liver of control and infected rats is shown in Table 4. As in perfused liver samples (Table 1), total water content also increased significantly in nonperfused livers of infected rats (Table 4).

There was a significant increase in intracellular water, with no significant change in extracellular water. The increase in intracellular water is consistent with increases reported previously.[2] There were no significant differences among control and infected rats with respect to total, intracellular, or extracellular Na^+ concentration, when results were expressed as milliequivalents of electrolyte per kilogram of fat-free wet tissue. However, when the results were expressed per kilogram of fat-free dry weight, a significant increase in total Na^+ concentration was noticed. Similar results were observed for total Cl^-, with its concentration increasing significantly in the livers of infected rats; however, total liver K^+ concentration decreased significantly. There was a significant increase in extracellular K^+ and a significant decrease in intracellular K^+. Intracellular K^+ even decreased when it was expressed per kilogram of intracellular water. Change in the percentage of plasma water during infection was not observed.

Effect of infection on the measured diameter and calculated volume of hepatocytes is shown in Table 5. There was a significant increase in the diameter and volume of the hepatocyte. Values for the volumes reported in Table 5 are only approximations, because hepatocytes were assumed to be spherical for the purposes of calculation. The stellate reticuloendothelial (Kupffer) cells of the infected liver did

TABLE 3—Effect of *S pneumoniae* Infection of Ten Rats on Chemical Constituents of Hepatic Dry Weight

Total liver	Controls (n = 5)	Infected (n = 5)	P
Dry weight (g)	2.27 ± 0.045	2.68 ± 0.07	< 0.001
Protein (g)	1.26 ± 0.03	1.60 ± 0.08	< 0.001
	(55.6 ± 1.2)	(59.7 ± 1.5)	NS
Lipid (g)	0.377 ± 0.029	0.493 ± 0.024	< 0.01
	(16.5 ± 1.3)	(18.5 ± 1.2)	NS
RNA (g)	0.117 ± 0.008	0.176 ± 0.004	< 0.001
	(5.2 ± 0.38)	(6.6 ± 0.16)	< 0.005
Carbohydrate (g)	0.067 ± 0.004	0.114 ± 0.008	< 0.001
	(3.0 ± 0.16)	(4.3 ± 0.33)	< 0.005
DNA (g)	0.014 ± 0.0004	0.015 ± 0.0007	NS

Data expressed as mean ± SE. No. in parentheses are percentages of dry weight. NS = not significant. n = No. of rats.

TABLE 4—Water Content and Electrolyte Concentrations in the Liver of Control and *S pneumoniae*-Infected Rats

Variable	Controls (n = 6)	Infected (n = 6)	P
$(H_2O)_T$ (ml/kg, FFWT)	714.1 ± 4.09	749.5 ± 4.37	< 0.001
$(H_2O)_I$ (ml/kg, FFWT)	519.0 ± 6.80	542.8 ± 10.5	< 0.05
$(H_2O)_E$ (ml/kg, FFWT)	195.0 ± 6.18	206.7 ± 8.76	NS
$(Na)_T$ (mEq/kg, FFWT)	29.69 ± 0.58	31.01 ± 0.77	NS
$(Na)_I$ (mEq/kg, FFWT)	2.84 ± 1.07	2.20 ± 0.76	NS
$(Na)_I$ [mEq/kg, $(H_2O)_I$]	5.38 ± 2.00	3.91 ± 1.33	NS
$(Na)_E$ (mEq/kg, FFWT)	26.85 ± 0.78	29.14 ± 1.37	NS
$(Na)_T$ (mEq/kg, FFDT)	104.1 ± 3.22	123.9 ± 3.19	< 0.005
$(Cl)_T$ (mEq/kg, FFWT)	27.7 ± 0.80	27.4 ± 0.89	NS
$(Cl)_T$ (mEq/kg, FFDT)	97.0 ± 3.38	109.5 ± 3.75	< 0.05
$(K)_T$ (mEq/kg, FFWT)	99.12 ± 1.70	93.35 ± 1.77	< 0.05
$(K)_I$ (mEq/kg, FFWT)	98.4 ± 1.69	92.4 ± 1.79	< 0.05
$(K)_I$ [mEq/kg, $(H_2O)_I$]	189.9 ± 5.30	170.7 ± 6.16	< 0.05
$(K)_E$ (mEq/kg, FFWT)	0.71 ± 0.02	0.99 ± 0.07	< 0.005
$(K)_T$ (mEq/kg, FFDT)	346.7 ± 3.92	313.4 ± 7.30	< 0.01
Plasma H_2O (%)	93.3 ± 0.21	93.2 ± 0.31	NS

Data expressed as mean ± SE. n = No. of rats; T = total; I = intracellular; E = extracellular; FFWT = fat-free wet tissue; FFDT = fat-free dry tissue; NS = not significant.

TABLE 5—Effect of *S pneumoniae* Infection in Six Rats on Diameter and Volume of Hepatocytes

Variable	Controls (n = 3)	Infected (n = 3)	P
Diameter (μm)	17.68 ± 0.30	21.07 ± 0.25	< 0.001
Volume (μm^3)	3039 ± 153	5259 ± 207	< 0.001

Data expressed as mean ± SE. Diameters were determined on 25 hepatocytes/rat and were averaged. Volumes were calculated by the equation $V = \pi d^3/6$ and were averaged.

not appear to be appreciably more numerous or prominent. Mitotic activity of the hepatocytes seemed equal in control and infected tissues. Lancet-shaped, gram-positive diplococci, although not prevalent, could be found mainly in stellate reticuloendothelial cells from all the infected livers, but not from the control livers.

Discussion

During *S pneumoniae* infection in the rat, there was a significant increase in liver weight. As previously reported,[12] control and infected rats fasted for 40 hours lost the same amount of weight. The increased liver weight in infected rats, therefore, resulted in an increase in the ratio of liver weight to body weight. Although an increase in total water was clearly demonstrated, water accumulation in the liver was limited to the intracellular compartment, with no significant change in the extracellular component. The increased hepatic water appeared to result from a shift of Na^+ and K^+ ions across liver cell plasma membranes. The movement of water and Cl^- follows passively with Na^+, according to the principles of bioelectrical balance and osmosis. The significant increases in total liver Na^+ and Cl^- concentration (in terms of fat-free dry tissue, rather than wet) indicates that a simultaneous increase in water content may mask changes in Na^+ and Cl^- concentrations when these values are expressed in terms of fat-free wet tissue or per liter of intracellular water. It is possible that the integrity of the liver cell plasma membrane had been modified during infection and that an accumulation of intracellular Na^+ in exchange for K^+ occurred. The proposed theory of membrane alterations is supported by the report of altered enzyme composition of plasma membranes isolated from rat liver after exposure to *S pneumoniae*.[5]

The significant increase in liver weight during *S pneumoniae* infection was due to increased intracellular water and increased dry weight. The increase in water content of the liver represented 84% of the increase observed in total liver weight. However, this was an expected result, because the major component by weight of the liver is water and represents 75% to 80% of the liver weight in controls[27] (Table 2). The increase in dry weight represented 16% of the increase observed in total liver weight. Every measured constituent of the dry weight, except DNA, increased significantly during infection. The unchanged DNA values indicate that cell division or mitotic activity was not altered during infection. All biochemical determinations were made in perfused and nonperfused livers. A significant increase in total liver water content and dry weight was observed in infected animals in perfused and nonperfused livers. Because blood components contained in the circulatory system of the liver could contribute to its dry weight and chemical composition, these measurements are reported for perfused livers.

There was a significant increase in the synthesis of secretory proteins by the liver during *S pneumoniae* infection.[12] The increased total dry weight of the liver could be the result of increased intracellular secretory protein, because measured dry weight would be expected to include weight contributed by secretory protein in transport through the liver cell. The increased total protein, lipid, and carbohydrate concentrations could also be explained by increased synthesis of secretory proteins, because these proteins are mainly glycoproteins and lipoproteins. The increased total RNA could reflect increased message for the synthesis of secretory protein.

The increase in the total dry weight of the liver during infection also could be due partly to an increase in the synthesis of the liver cell itself. An increase in the synthesis of the liver cell plasma membrane during *S pneumoniae* infection has been observed,[h] and possibly the synthesis of other intracellular organelles involved in the synthesis, transport, packaging, and secretion of plasma proteins by the liver is increased.[12] In addition, the specific activity of several liver enzymes increased significantly during *S pneumoniae* infection.[5,10] This increase in specific activity could occur by increased synthesis of liver enzymes or by other mechanisms.

The increase in the measured hepatocyte diameter may

[h] Little JS, Department of Clinical Investigation, Madigan Army Medical Center, Tacoma, Wash: Unpublished data, 1980.

result only from increased intracellular water and swelling, or it may reflect a larger cell size due to increased synthesis of the liver cell itself. The increased liver cell plasma membrane synthesis and measured dry weight are consistent with increased synthesis of the liver cell.

Because infected rats were anorectic, it was necessary to fast control and infected animals. Because fasting by itself caused a significant decrease in the total dry weight and all measured constituents of the dry weight, except DNA,[1] it could be argued that our results do not reflect increased synthesis of any constituent during infection, but simply less of a decrease after fasting. This possibility does not seem likely, because there was a significant increase in the incorporation of labeled precursors into liver RNA,[7,28] protein,[2,6,12] and lipid[10,29] during *S pneumoniae* infection, indicating that synthesis of these constituents was actually increased.

Different results were obtained when values were expressed per gram of liver and per total liver. Certain measured constituents showed no change or showed decreases when the results were expressed per gram of liver because of the dilution effect caused by the increased water content of the liver during infection. It is important to be aware of this increased water content when expressing biochemical results for the liver. Incorrect conclusions could be drawn if all biochemical values were expressed per gram of liver instead of per total liver weight.

[1] Little JS, Rill WL, Department of Clinical Investigation, Madigan Army Medical Center, Tacoma, Wash: Unpublished data, 1980.

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